



Further, the DNA is useful for production of the above described enzyme, or serves as a therapeutic tool, diagnostic tool or research tool for the treatment of diseases caused by the abnormal expression of Gb3/CD77, or it may be useful for the treatment or diagnosis of diseases involved in the action of verotoxins.

[0090]

[Sequence Listing]

SEQUENCE LISTING

<110> Seikagaku Corporation

Koichi Furukawa

<120>  $\alpha$ 1,4-GALACTOSYLTRANSFERASE AND DNA ENCODING THEREOF

<130> P-7040

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<141> 2000-02-14

<160> 2

<170> PatentIn Ver. 2.0

[0091]

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Pro Glu Glu Leu Pro Arg Leu Leu Ser Ala Thr Tyr Ala Val His Val  
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Met Lys Met Tyr Leu

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Cys Arg Gly Val Thr Thr Leu Pro Pro Glu Ala Phe Tyr Pro Ile Pro			
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Trp Gln Asp Trp Lys Lys Tyr Phe Glu Asp Ile Asn Pro Glu Glu Leu			
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Pro Arg Leu Leu Ser Ala Thr Tyr Ala Val His Val Trp Asn Lys Lys			
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Ser Gln Gly Thr Arg Phe Glu Ala Thr Ser Arg Ala Leu Leu Ala Gln			
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Leu His Ala Arg Tyr Cys Pro Thr Thr His Glu Ala Met Lys Met Tyr			
	340	345	350
Leu			

[Brief Description of Drawings]

[Fig. 1] It shows flow cytometry indicating the expression of Gb3/CD77 by L cells. The left diagram relates to L cells transfected with pCDM8 while the right diagram to L cells transfected with pVTR1/CDM8. The thick line indicates the result of cells stained with mAb38.13 and FITC-conjugated rabbit anti-rat IgG (secondary antibodies) while the thin line the result of cells stained only with

the secondary antibodies (control).

[Fig. 2] It shows TLC charts of glycolipids extracted from cells transiently transfected with  $\alpha 1,4$  Gal-T gene.

A: TLC of glycolipids extracted from L cells transfected with pCDM8 (VC) or pVTR1/CDM8 (TF). RBC represents neutral glycolipids extracted from human B red blood cells.

B: TLC immunostaining of Gb3/CD77 by mAb38.13.

[Fig. 3] It shows the hydropathy plot of a polypeptide of the present invention.

[Fig. 4] It shows the  $\alpha 1,4$  Gal-T activity in the extracts of transient transfectants of pVTR1.

A:  $\alpha 1,4$  Gal-T activity when LacCer was used as an acceptor.

B:  $\alpha 1,4$  Gal-T activity when various acceptors were used. PG represents paragloboside.

[Fig. 5] It shows the result of northern blotting of  $\alpha 1,4$  Gal-T gene.

A: the upper columns show the results of hybridization with a  $^{32}\text{P}$ -labeled probe derived from pVTR1, while the lower columns show the results of hybridization of the same membranes as in A with a  $\beta$ -actin cDNA probe(control).

B: the expression levels of mRNA of  $\alpha 1,4$  Gal-T gene were compared among various human tissues. The ordinate represents the percentage of the expression level of a given tissue with respect to the level of heart after correction with the control.

[Fig. 6] It shows flowcytometry of stable transfectant cells.



The left diagram relates to cells transfected with pSV2neo while the right diagram to cells transfected with pVTR1 and pSV2neo. The thin line indicates the number of cells stained with mAb38.13 and FITC-conjugated rabbit anti-rat IgG (secondary antibodies) while the thick line the number of cells stained only with the secondary antibodies (control).

[Fig. 7] It shows the results of MTT assay of L-neo and L-VTR1. The left graph shows the result of L-neo while the right one the result of L-VTR1.

[Fig. 8] It shows the effect of vero toxins on the cell growth.

[Fig. 9] It shows an electrophoresis indicating the result of DNA fragmentation assay.

[Document Name] Abstract

[Object] To provide  $\alpha$ 1,4-galactosyltransferase to transfer a galactose residue to C4 position of galactose residue of lactosylceramide or galactosylceramide, and DNA coding for the enzyme.

[Solving Means]

The following polypeptides (A) and (B), and DNAs encoding thereof:

(A) a polypeptide consisting of an amino acid sequence represented by the amino acid Nos. 46-353 in SEQ ID NO: 2; or

(B) a polypeptide which comprises an amino acid sequence including substitution, deletion, insertion or transposition of one or few amino acids in the amino acid sequence of (A) and which has an enzymatic activity to transfer a galactose residue from a galactose donor to C4 position of galactose residue of lactosylceramide or galactosylceramide which serves as an acceptor.

[Drawing Selected] Fig.1